Determination of Color, Pigment, and Phenolic Stability of Non-Acylated Anthocyanins from *Berberis boliviana* L. in Yogurt Systems

Taylor C. Wallace and M. Monica Giusti

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, OH 43210

Abstract

As natural food products and colorants are on the verge of a new era, anthocyanins are of particular interest because of their known antioxidant power, beautiful color, and relative stability in high acid foods. This study focuses on the Peruvian berry *Berberis boliviana*, rich in phenolics and non-acylated anthocyanins (9 and 7% dry weight, respectively)

Our objective was to determine color and pigment stability using different levels of *Berberis boliviana* whole fruit addition in comparison to purple carrot acylated anthocyanin extracts throughout various yogurt fat matrices.

Yogurt samples with 0, 2, and 4% fat contents were colored using 10 and 20mg of *Berberis boliviana* anthocyanins. Yogurt samples were compared with FD&C Red 40, Betalaines, and Purple Carrot anthocyanin treatments, along with a negative control yogurt sample (no color added). All treatments were stored at 4ºC for 1, 7, 14, 30, 45, and 60 days. Yogurt color (CIELab, chroma, and hue angle) was measured with a Hunter ColorQuest. Anthocyanin and phenolic degradation kinetics was evaluated using the pH differential method and Folin Ciocalteau methods, respectively. Qualitative changes were monitored by HPLC coupled to a MS and PDA detectors.

Initial color of the yogurt treatments containing *Berberis boliviana* anthocyanins showed very similar color characteristics (L=65, a*=12, b*=-6, chroma=14, and hue angle=335º) to commercial blueberry yogurt (L=65, a*=10, b*=-3.5, chroma=10.5, and hue angle=341º) colored with FD&C Red 40 and Blue 1. Color and pigment stability was achieved in both acylated and non-acylated anthocyanins throughout the study and was related to the fat content of the matrix. The added stability of acylated pigments did not result in a marked difference throughout the shelf life of the yogurt (typically 30 days).

Addition of *Berberis boliviana* whole fruit to yogurt resulted on an attractive and marketable added value product with acceptable stability for commercial applications.

Objectives

- To create a value added, stable, high antioxidant containing, and nutritious functional food product free of synthetic colorants.
- To determine color, pigment, and phenolic stability as well as the degradation kinetics using contrasting levels of *Berberis boliviana* L. pigment / whole fruit addition to the various fat matrices of yogurt.
- To compare the non-acylated Anthocyanin treatments to ones containing betalaines, FD&C Red 40, and acylated purple carrot anthocyanins.

Background

Anthocyanins are flavonoid compounds responsible for the purple to red to blue colors in many flowers and berries. Research suggests that anthocyanins contain high antioxidant effects and may help aide in the treatment of chronic diseases such as coronary heart disease and cancer (Wrolstad 2004). Anthocyanins may help increase visual acuity and cognitive functions (Wrolstad 2004). Non-Acylated anthocyanins typically exhibit less stability in foods but contain greater antioxidant properties. Typical berries native to the U.S contain between .1 and .5 % of anthocyanins on a dry weight basis. The non-acylated anthocyanins from *Berberis boliviana* L. have recently been characterized for their high concentration of pigment (<7.0%) and thus eliminate the need for costly industrial extraction and concentration (Del Carpio Jimenez and coworkers, 2006).

Methods

Creation of a Whole Berry Powder

Berberis boliviana whole berries were seeded and frozen in a crucible using liquid nitrogen. Berries were then blended for 15 seconds using a Waring Commercial Laboratory blender (Waring Laboratory Science, Torrington, CT) with a stainless steel container before addition to the yogurt samples. Sample berry powder was stored at -10ºC to decrease clumping and water migration in the storage container.

Color Treatments

Yogurt samples were divided into six treatments at three fat levels per treatment (0, 2, and 4% fat respectively). Treatments 1 and 2 were colored with 10 and 20mg of non-acylated anthocyanins measured as cy-3-glu equivalents by the pH differential method from the whole fruit addition of the Peruvian berry *Berberis boliviana*. Treatment three was colored with 10 mg purple carrot acylated anthocyanin extracts which were hypothesized to have increased stability due to interaction between the pigment's acid groups and fat molecules in yogurt. Treatment 4 contained Betalaines at an initial color value of 9.8 intensity. Treatment 5 contained 200 parts per million of FD&C Red 40 per 100 grams of yogurt. Treatment 6 acted as the control group for the experiment and contained no color additives.

Storage Study

The storage time was 2 months during which color, pigment, and phenolic degradation data points were collected every week for the first 2 weeks and every other week after that. Samples were kept under normal refrigerated conditions at 40ºC at all times.

Colorimetric Analysis

Each colored yogurt sample was transferred to a 5cm path length cell and read for CIELab, chroma, and hue angle values using a Hunter ColorQuest XE (Hunter Labs, Reston, VA). Samples were read with a 1 inch opening in triplicate using reflectance specular included and a 10 degree observer angle. The colored yogurt samples were compared to commercial brand yogurt for color equivalency.

Extraction of Pigments from the Yogurt Complex:

Ten grams of the colored yogurt sample was blended with 30mL of .1% HCl acidified methanol for two minutes on low using a Waring Commercial Laboratory blender (Waring Laboratory Science, Torrington, CT). Samples were then placed into 50mL centrifuge tubes and centrifuged at 3500 rpm for 15 minutes using a Beckman J2-21M Induction Drive Centrifuge (Feakes and Giusti 2003). The resulting supernatant was concentrated at 40ºC with a Buchi Rotoevaporator and brought to a known volume of 25mL with distilled deionized water. The final extracts were used for spectrophotometric and HPLC analysis.

Spectrophotometric Analysis

Monomeric anthocyanin content, were determined using the pH differential method (Giusti and Wrolstad 1999). A UV-Visible Spectrophotometer (Shimadzu, Columbia, MD) was used for spectral measurements at 420, 520, and 700 nm with 1 cm pathlength disposable cells. Pigment content was calculated as cyanidin-3-glucoside, using a molecular weight of 449.3 and an extinction coefficient of 26,000 L cm-1 mg-1 (Giusti and Wrolstad 1999). Total phenolic degradation was evaluated using the Folin Ciocalteau method and calculated as gallic acid equivalents using the UV-Visible Spectrophotometer (Shimadzu, Columbia, MD) as described by Giusti and Wrolstad (2001).

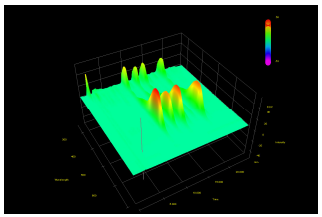

Figure 1: Spectral view of the 10 non-acylated anthocyanins of Berberis boliviana L.

HPLC Analysis

Samples were analyzed using a HPLC (Shimadzu, Columbia, MD) coupled to an electro-spray mass spectrophotometer (Shimadzu, Columbia, MD) and photodiode array (Shimadzu, Columbia, MD) detectors. Separation was achieved with a Symmetry C-18 column (Waters, Montreal, Quebec) using a linear gradient system of A: 5% formic acid in water and B: 100% acetonitrile. B was increased from 7 to 15 percent over 22 minutes. The flow rate was set at .8mL/min and an injection volume of 50µL. Spectral data of the anthocyanins was collected at 520nm.

Results

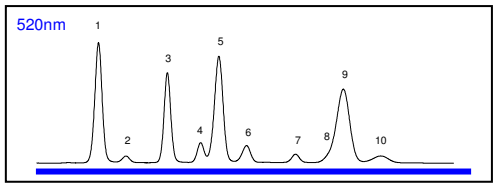

Figure 2. The ten anthocyanin peaks of *Berberis boliviana* L in the following sequence: Delphinidine-3-glucoside (1), Delphinidin-3-rutinoside(2), Cyanidin-3-glucoside(3), Cyanidin-3-rutinoside (4), Petunidin-3-glucoside (5), Petunidin-3-rutinoside (6), peonidin-3-glucoside(7), peonidin-3-rutinoside (8), malvidin-3-glucoside (9), and malvidin-3-rutinoside (10).

Color Values	Commercial yogurt	*Berberis boliviana*	FD&C Red 40	Betalaines	Purple Carrot
L*	65	65	71	80	64
a*	10	12	35	11	13
b*	-3.5	-6	4	1	-5
Chroma	10.5	14	35	11	14
Hue Angle	341°	335°	6°	3°	340°

Table 1. The color characteristics of *Berberis boliviana* L. pigments at 20mg/100g of yogurt showed the closest resemblance to the Yoplait Blueberry Yogurt.

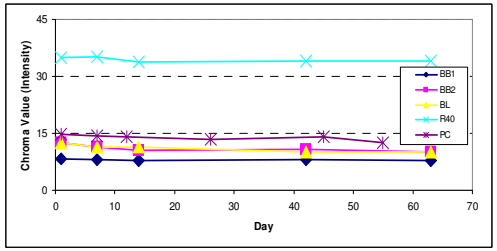

Figure 3. Chroma values over the 2 month storage time exhibited stability. BB = *Berberis boliviana* L. anthocyanins , BL = Betalaines, R40 = FD&C Red 40, and PC = Purple Carrot anthocyanin extract.

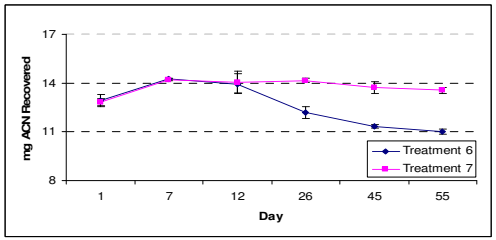

Figure 4. Monomeric anthocyanin degradation over 2 months of storage. Treatment 6 represents the non-acylated anthocyanins from *Berberis boliviana* L. Treatment 7 represents the more stable acylated anthocyanins from Purple Carrot extracts.

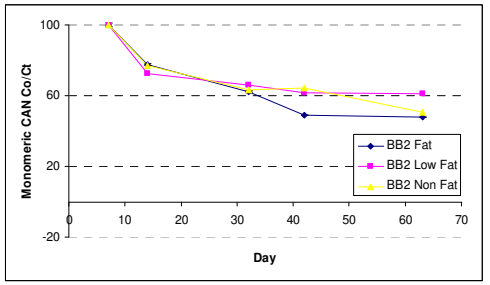

Figure 5. *Berberis boliviana* L. treatment 2 anthocyanin addition to various fat yogurt fat matrices over the 2 month storage period. BB = *Berberis boliviana*

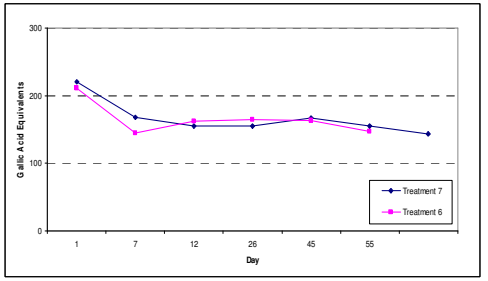

Figure 6. Total phenolic degradation over the 2 month storage period. Treatment 6 represents the non-acylated *Berberis boliviana* L. anthocyanins. Treatment 7 represents the acylated Purple Carrot anthocyanins.

Conclusion and Discussion

- Addition of grinded whole berries (*Berberis)* rich in anthocyanins achieved color characteristics similar to commercial brand yogurt with acceptable stability.
- Acylation made the anthocyanin molecule more stable throughout the storage study.
- Stability of acylated and non-acylated anthocyanins between the various fat matrices in yogurt was not statistically different.
- A value added functional food product with enhanced phenolic compounds was created. An increase in the stability of anthocyanin compounds in low acid foods was also accomplished.

Acknowledgments and References

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